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TOXICITY OF 1,1'-ALKYL-4,4'-BIPYRIDYLIUM SALTS IN THE RAT

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ABSTRACT

Methyl (paraquat), propyl, isopropyl, butyl, methyl-pentyl, hexyl, octyl and benzyl viologens (1,1'-Alkyl-4,4'-bipyridylium salts) were administered subcutaneously to female Sprague-Dawley rats to determine relative toxicities. These compounds all produce the spectrum of effects previously reported for paraquat and additionally produce a focal necrosis at the injection site, non-emptying of the stomach and adrenal enlargement. A lethal dose of propyl, hexyl or benzyl viologen often produced a yellow to red serous fluid in the pleural cavity. Many of the signs observed with viologen poisoning are similar to adrenal hormone effects and the suggestion is made that the adrenals may be contributing to toxicity.

INTRODUCTION

The utility of methyl viologen (1,1'-Dimethyl-4,4'-bipyridylium dichloride) as a redox indicator for biological systems was first suggested in 1933 by Michaelis and Hill.[1] Since 1963 this compound has been sold as the contact herbicide, paraquat (PQ). When ingested or injected methyl viologen produces lung pathology in many laboratory animals and humans that is characterized by edema and hemorrhage followed by a rapidly progressing intra or inter alveolar fibrosis.[2,3,4,] It has been speculated that only the

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methyl homologue of the viologen series was capable of producing lung hemorrhage and fibrosis.[5] Others have examined the lung effects of benzyl viologen without testing lethal dose or pathology in other organs.[6] The present studies examined gross pathological changes produced in rats by several homologues of the viologen series.

MATERIALS AND METHODS

Propyl, isopropyl, butyl, methyl-pentyl, hexyl and octyl viologens were synthesized as iodide salts.[7] Methyl and benzyl viologen (chloride salts) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Gallard-Schlesinger Chem. Mfg. Corp. (Carle Place, NY) respectively. The compounds were dissolved in distilled water and administered subcutaneously (sc) to female Sprague-Dawley rats (216-286 g; Simonsen Laboratories, Gilroy, CA). Two animals were injected sc in the right thigh at each dose level. Dosages were increased geometrically (1.5x) within the experimental range. Controls received distilled water sc. Rats were allowed free access to feed and water while housed in plastic shoe box cages. Rats were observed daily for indications of behavioral and physical abnormalities.

All rats were necropsied at death to observe gross pathological changes. Animals that did not die spontaneously were killed when their weights approached predosing levels (11-17da). They were anesthetized with ether vapor and exsanguinated prior to gross pathological examination. Protein levels of pleural exudates were determined using a bovine serum albumin standard.[8] Adrenals were kept hydrated in isotonic saline prior to dissection of adhering fat and weighed (± 0.1 mg) following blot drying.

RESULTS

Illustrated in Table 1 are responses observed following lethal and sublethal sc dosing of the viologens in female rats. Lowest lethal dose (LLD) was defined as that sc dose which produced death in both animals within 11 days. When doses larger than the LLD were given (hexyl, butyl and benzyl viologen), death occurred in less time than following the LLD.

Lethal doses of viologens produced a common set of signs in rats. Signs usually included piloerection initially around the scapulae which rapidly generalized (within 24 hr) to the whole body. Piloerection was not observed with any dose of octyl viologen. Weight loss (except in the case of hexyl viologen where the body weight increased in all but the highest dose given) was observed with all the other viologens. A marked swelling of the right thigh developed around the site of injection. Dyspnea and a general lack of both mobility and exploratory behavior were also common. These signs were observable within 24 hr of dosing. Some animals also developed chromodacryorrhea and reddish brown exudate around the nares. Time to onset of these signs varied from 24-96 hr.

A lethal dose of any of the viologens also produced extensive hemorrhaging of the lungs. None of the lungs sank in water, although they floated lower in the water than controls. Onset of

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TABLE 1

Dosage (μmole/kg)	Time to death (hr)[a]	Adrenal Wt (mg)[b]	Pleural Fluid (ml)	ΔBody Wt (g)[c]
		Methyl Viologen (PQ)		
105	72,168	(49.9,47.1)[d]	---------[e]	-28,-43
70	11 da	30.5,23.9	---------	+15,+35
47	11 da	31.3,39.7	---------	+22,+34
		Propyl Viologen		
351	45,39	(49.4,43.7)[d]	1.7,3.4	-18,-17
234	17 da	NR[f]	---------	-14,+14
156	17 da	NR[f]	---------	-7,+14
		Butyl Viologen		
188	24,32	(36.7,57.1)[d]	---------	-26,-24
125	72,192	(48.9,48.0)[d]	-----,1.2	-26,-113
83.3	17 da	NR	---------	+30,+26
		Methyl-Pentyl Viologen		
55.5	120,73	(53.4,54.6)[d]	---------	-59,-53
37.0	17 da	28.9,36.9	---------	-13,+47
24.7	17 da	30.3,29.8	---------	- 6,+21
		Hexyl Viologen		
125	18,20	NR	NR	- 8,-12
83.3	60,36	NR	NR	+14,+ 6
55.5	48,72	NR	5.0,6.8	+18,+18
37.0	48,37	(48.6,43.7)[d]	10.0,6.2	+ 3,+20
24.7	13 da	29.9,33.2	---------	+ 2,+ 6
		Octyl Viologen		
281	28,72	(51.4,54.2)[d]	---------	-24,-31
188	15 da	NR	---------	+ 1,+17
125	15 da	NR	---------	NR
		Benzyl Viologen		
40.0	<21	42.3,30.0	1.5,-----	NR
26.8	<21	(42.8,34.9)[d]	1.5,2.2	NR
17.8	14,17	(45.1,48.6)[d]	3.2,2.8	+ 2,- 1
11.9	192, 11 da	(54.8,37.1)[d]	---------	-63,+ 1

[a] Time to death when shown in days denotes sacrifice because animal appeared to have recovered.

[b] Mean weight of both adrenals from each rat.

[c] Change in body weight from predosing level.

[d] Significantly heavier than controls $p < 0.05$.

[e] --------- = Not observed.

[f] NR = Not measured.

hemorrhaging was rapid; it was readily observable in rats that died <21 hr following treatment with benzyl viologen. The pleural cavity of rats that died following propyl, hexyl or benzyl viologen treatment were often filled (1.5 - 10 ml) with a clear yellow to red serous fluid which contained 20.8 ± 1.5 mg/ml; range 12.0 - 27.5 mg/ml; n = 10 protein. One animal treated with butyl viologen (125 μmol/kg) had a small amount of pleural fluid. Pleural cavities of rats succumbing to methyl, methyl-pentyl, and octyl viologen were similar to controls, i.e. no fluid could be recovered by aspiration.

Stomachs and cecae of all viologen dosed animals that died were full (sometimes distended) with food, while the small intestines were quite empty except for occasional gaseous bloating. Following butyl viologen, two rats (one each from the two lethal dose levels) had large areas of hemorrhaging in the small intestine. Adrenals of all animals that died spontaneously appeared larger, and were significantly heavier than controls (27.6 ± 1.3 mg; range 20.1 -35.1 mg; n = 10). Adrenal weight increase was observed even in animals dying 14-18 hr following benzyl viologen treatment. Adrenals were also enlarged in animals sacrificed two weeks following sublethal viologen dosing although this trend was not statistically significant. Adrenals of controls were unremarkable.

Sublethal dosages produced no signs of lung hemorrhage when the animals were killed 11-17 days later but there were signs of fibrotic consolidation. These lungs did not sink in water. Of all sublethally treated animals, those treated with propyl viologen (156 and 234 μmol/kg) showed the most distinctive gross lung pathology. This consisted of convolutions of the lung surface whose troughs were of a lighter color, denser and harder to cut than other

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areas. The other compounds produced scattered areas of consolidation that appeared as light grey indentions on the lung surface (1-3 mm) with no preferential localization. Hair loss was noticeable on the dorsal portion of the heads of some animals (especially those treated with methyl, propyl and butyl viologen) one week following dosing and became progressively greater extending past the scapulae after two weeks. Focal necrosis (ca. 5-7 mm) developed on the skin at the site of injection regardless of the homologue or dose given (except with isopropyl viologen) if the animal survived more than four days. This necrosis was characterized by hair loss around the perimeter and formation of a hard scab with no adhesion to underlying muscle tissue. Control rats did not develop necrosis at the injection site. Isopropyl viologen (not shown in Table 1) had no apparent ill effects on rats at 185 µmol/kg. A sufficient quantity of isopropyl viologen was not available to attain a lethal dose. A summary of estimated lethality in terms of µmol/kg and mg/kg with molecular weights of the compounds is presented in Table 2.

DISCUSSION

The viologens tested were qualitatively similar in their effects. The dose required to produce these effects varied over a wide range depending upon the compound; however, the LLD placed the homologues in the following relative order of potency: isopropyl < propyl < octyl < butyl < methyl < methyl-pentyl < hexyl < benzyl. At a lethal dose, all of the viologens tested produced lung hemorrhaging and edema as evidenced by increased density in water. The

TABLE 2

Summary of Lethal Doses and Molecular Weights of Bipyridyls

Viologen[a]	Molecular Wt.	Lowest Lethal Dose[b] µmol/kg	Lowest Lethal Dose[b] mg/kg
Methyl[c]	257.2	105	27
Propyl	496.2	351	174
Isopropyl	496.2	>185	>92
Butyl	524.2	125	66
Methyl-Pentyl	496.2	56	28
Hexyl	580.3	37	21
Octyl[d]	542.4	281	152
Benzyl[c]	409.4	18	7.4

[a] Anion was iodide except where noted otherwise (c,d).
[b] Lowest lethal dose is that which produced death in both animals within 11 days following sc dosing.
[c] Chloride salt.
[d] Bromide salt.

overall objective was to find the approximate relative toxicity of these compounds rather than to statistically determine LD_{50}s. Because the lethality curve for methyl viologen is extremely steep (LD_{10} to LD_{90} requires only a 2.5 fold intraperitoneal dose increase;[9] LD_{25} to LD_{90} = 1.3 fold sc dose increase, L. L. Smith, of Imperial Chemical Industries, Ltd.) the values for LLD are probably useful estimates of the lethality of these compounds. With the exception of benzyl viologen, a 1.5-fold increase in dose made the difference between no deaths and the deaths of both animals.

Certain toxic effects of the viologens in rats have precedent in human PQ poisonings. The presence of a yellowish serous fluid in the pleural cavity of humans that have died from PQ poisoning has been observed.[10,11] Although we did not observe this fluid

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in PQ treated animals, it was often seen in rats receiving a lethal dose of benzyl, hexyl and propyl viologen. None of these compounds consistently produced pleural fluid at the lethal doses tested, however. Although the lung sustains extensive damage following PQ poisoning and appears to be a target organ, it is known that other vital organs including heart, liver, kidney and adrenals can also be affected in human PQ poisoning.[2,12] Noteworthy is the correlation between blood flow per unit tissue mass (Table 3) and pathology observed in these tissues following PQ poisoning. This observation suggests that those tissues receiving

TABLE 3

Blood Flow to Human Tissues[a]

Tissue	% Body weight	% Cardiac output	Blood flow
			ml/100g/min
Adrenals	0.02	1	550
Kidneys	0.4	24	450
Lung	2.2[b]	98[a]	420
Thyroid	0.04	2	400
Liver hepatic	2	5	20
portal		20	75
Portal-drained viscera	2	20	75
Heart (basal)	0.4	4	70
Brain	2	15	55
Skin	7	5	5
Muscle (basal)	40	15	3
Connective	7	2	1
Fat	15	2	1

[a] Adapted from Guyton.[13]
[b] Calculated from Warwick and Williams[14] assuming body weight = 50 kg.

highest blood flow (and thus PQ) develop pathology most frequently, and invites the question, "Does PQ cause thyroid damage?"

References to adrenal pathology in laboratory animals treated with PQ are sparse,[15,16] but there are several references to adrenal pathology in humans following a lethal exposure.[17,18,19,20] When PQ is injected into rats, it is concentrated in adrenals to levels in excess of plasma levels in a manner similar to lung and kidney.[21,22] Although in vitro PQ uptake by kidney cortex slices[23] and lung slices[24] has been demonstrated, it would be difficult to carry out such studies on rat adrenals since no more than two slices per adrenal could be realistically achieved. By analogy, the variability in PQ uptake rate by lung slices from one animal to another suggests there would be considerable variability among rats in adrenal uptake of PQ.

The signs of poisoning seen in rats, i.e., anorexia, renal insufficiency, pulmonary edema, myocardial necrosis, hyperexcitability (following supralethal doses),[2] changes in prostanglandin and blood glucose levels[25] piloerection and non-emptying of the stomach are all effects that could be attributed to high levels of circulating epinephrine.[26] The use of propranolol and other β-adrenergic blocking agents to reduce paraquat toxicity, and the increase in paraquat toxicity with β-adrenergic agonists such as isoproterenol[27,28] are consistent with the suggestion that adrenal involvement contributes to paraquat toxicity.

Maling et al.[28] studied the effects of propranolol in preventing the synergistic lethality produced by PQ and β_2-agonists. Their

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conclusion that the β_2-agonists produce synergism with PQ by reducing renal clearance of PQ is not at variance with the postulate that PQ itself is causing indirect adrenergic effects. To detect circulating levels of epinephrine or norepinephrine precisely requires radioimmunoassay which may help elucidate the mechanism of action but which is beyond the scope of these studies.

Adrenocorticotropic hormone levels may be elevated in rats receiving a lethal dose of PQ.[29] Toxic effects of PQ such as fluid retention in the gastrointestinal tract and hemoconcentration[30] as well as the pleural exudate might be a result of increased aldosterone secretion which affects fluid balance. Although PQ has been shown to affect glucocorticoid levels in rats,[29] the duration of change and levels of these and other hormones (e.g. epinephrine, norepinephrine and aldosterone) have not been reported. Paraquat by expressing an indirect action through the adrenals may change hormone levels that add to or result in toxic signs in lung that are considered ultimately responsible for most PQ deaths. Further experimentation to determine whether the adrenals are responding to the viologens, stress or are also contributing to overall symptomology should be undertaken.

The present work fills a gap in the knowledge of gross pathological effects of these compounds. Although they are all patented for herbicidal properties,[31] the mammalian toxicities have never been detailed with exception of PQ. Several of the viologens tested for toxicity in this section were purportedly synthesized and tested for toxicity previously by Russian investigators.[32]

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Unfortunately the Chemical Abstracts reference to this work is incorrect and has prohibited examination of the original article. However, the abstract does mention that parenteral minimum lethal doses for methyl (PQ), isopropyl, butyl, and hexyl viologen were in the range of 16-18 mg/kg in mice.

Methyl viologen is absorbed through the skin as evidenced by the lethality it produces in rats following skin painting.[33] In the course of synthesizing these compounds, inadvertent dermal contact with several viologens on the fingers were followed by a peculiar bitter taste on the back of the tongue within five minutes of exposure. Others of these compounds may be capable of dermal absorption.

Because of the extreme toxicity that some of the viologens produce in low doses, industrial applications of these compounds in film developer adjuvants[34,35,56] electroplating metal brighteners[37] and electrochromic displays[38,39] may present potentially hazardous occupational and even consumer exposure. The examples of new applications of these compounds are only a few of dozens cited in recent literature. Paraquat seems representative of viologen toxicity. It is toxic by every route tested such that inhalation, dermal contact or ingestion could present potentially lethal routes of exposure.[12] The toxicology of new violgen compounds for industrial application should be closely studied. Benzyl viologen, the most toxic of the compounds tested, has sc toxicity (LLD = 18 μmol/kg) comparable with rat oral toxicities of such insecticides as parathion (LD_{50} = 17 μmol/kg) and carbofuran (LD_{50} = 24 μmol/

kg).[40] The exposure of industrial personnel and consumers to viologens could result in poisonings whose etiology might be difficult to determine, since the lung effects are similar to diseases like pneumonia,[41] interstitial pulmonary fibrosis[42,43] and idiopathic respiratory distress syndrome.[44,45,46] Further complicating the problem of viologen toxicity is that there is no known antidote for viologen (paraquat) poisoning and the current method of treatment is supportive i.e., catharsis, hemoperfusion, adsorbents and stomach lavage.[47]

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